BLUE/WHITE SCREENING BY IPTG-XGal

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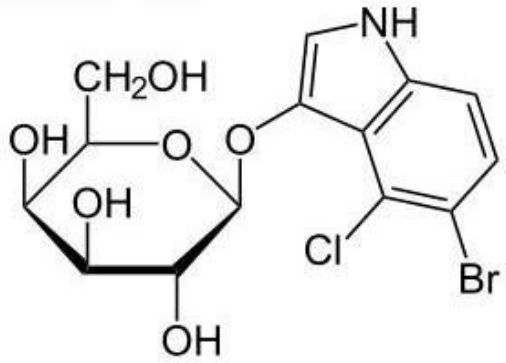
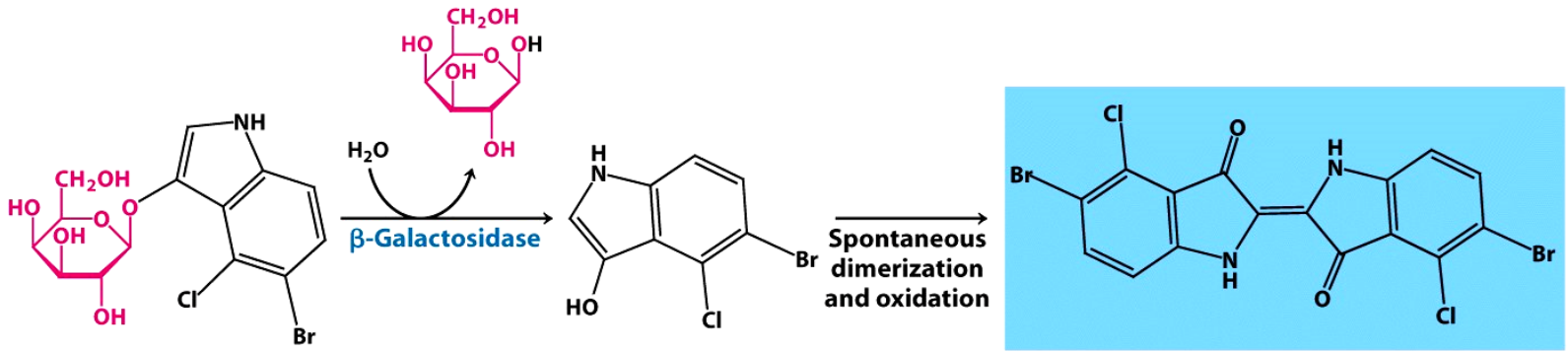
X-gal

- **X-gal** (5-bromo-4-chloro-indolyl-galacto-pyranoside) is an organic compound having galactose linked to a substituted indole.
- **X-gal is frequently used to test for the activity of the enzyme β-galactosidase**, as it is one of many indoxyl glycosides and esters that yield insoluble blue compounds similar to indigo as a result of enzymatic hydrolysis.
- **X-gal is an analogue of lactose**, and therefore may be hydrolyzed by the enzyme β-galactosidase which cleaves the β-glycosidic bond in D-lactose.



Use of X-gal for Detection of β-galactosidaese

- X-gal, when cleaved by β-galactosidase, yields galactose and 5-bromo-4-chloro-3-hydroxy-indole.
- The latter then spontaneously dimerizes and is oxidized into 5,5'-dibromo-4,4'-dichloro-indigo, an intensely blue product which is insoluble (2).
- **As X-gal itself is colourless, the presence of blue-colored product may be used as a test for the presence of an active β-galactosidase.**
- **This easy identification of an active enzyme allows the gene for β-galactosidase (the *lacZ* gene) to be used as a reporter gene in various applications.**



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α-complementation & Use in Gene Cloning

- In gene cloning, **X-gal is used as a visual indication** of expression of a functional β-galactosidase enzyme in a technique called blue/white screening. This method of screening is a convenient way **to distinguish a successful cloning product from other unsuccessful ones**.

- The blue/white screening method relies on the principle of **α-complementation** of the β-galactosidase gene, where **a fragment of the lacZ gene** (lacZα) **in the plasmid can complement another mutant lacZ gene** (lacZΔM15) in the bacterial cell. Each of these genes produces a non-functional peptide. However, **when expressed together in a cell** (when a plasmid containing *lacZα* is transformed into a *lacZΔM15* cells) **they form a functional β-galactosidase**. The presence of an active β-galactosidase may be detected when cells are grown in plates containing X-gal, the blue-colored product precipitated within cells resulted in the characteristic blue colonies.

- In the method of blue-white screening, the host *E. coli* strain carries the *lacZ* deletion mutant (*lacZΔM15*) which contains the ω-peptide, while the plasmid used as vector carries the *lacZα* sequence which encodes the first 59 residues of β-galactosidase, the α-peptide. When a plasmid containing the *lacZα* sequence is transformed into a *lacZΔM15* cells, the two peptides are expressed together and they form a functional β-galactosidase enzyme.

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Use in Gene Cloning [cont.]

- However, the plasmid also carries an internal multiple cloning site (MCS) within the *lacZα* sequence. This MCS within the *lacZα* sequence can be cleaved by suitable restriction enzymes so that foreign DNA may be inserted within the *lacZα* gene, thereby disrupting the gene. This disruption of *lacZα* gene results in loss of α-complementation. Consequently, in cells containing the plasmid with an insert, **no functional β-galactosidase can form** resulting in **white colonies**.

- Thus, blue colonies indicate that they contain a vector with uninterrupted *lacZα* **(therefore no insert)**, while white colonies, where X-gal is not hydrolyzed, indicate **the presence of an insert** in *lacZα* which disrupts the formation of an active β-galactosidase.

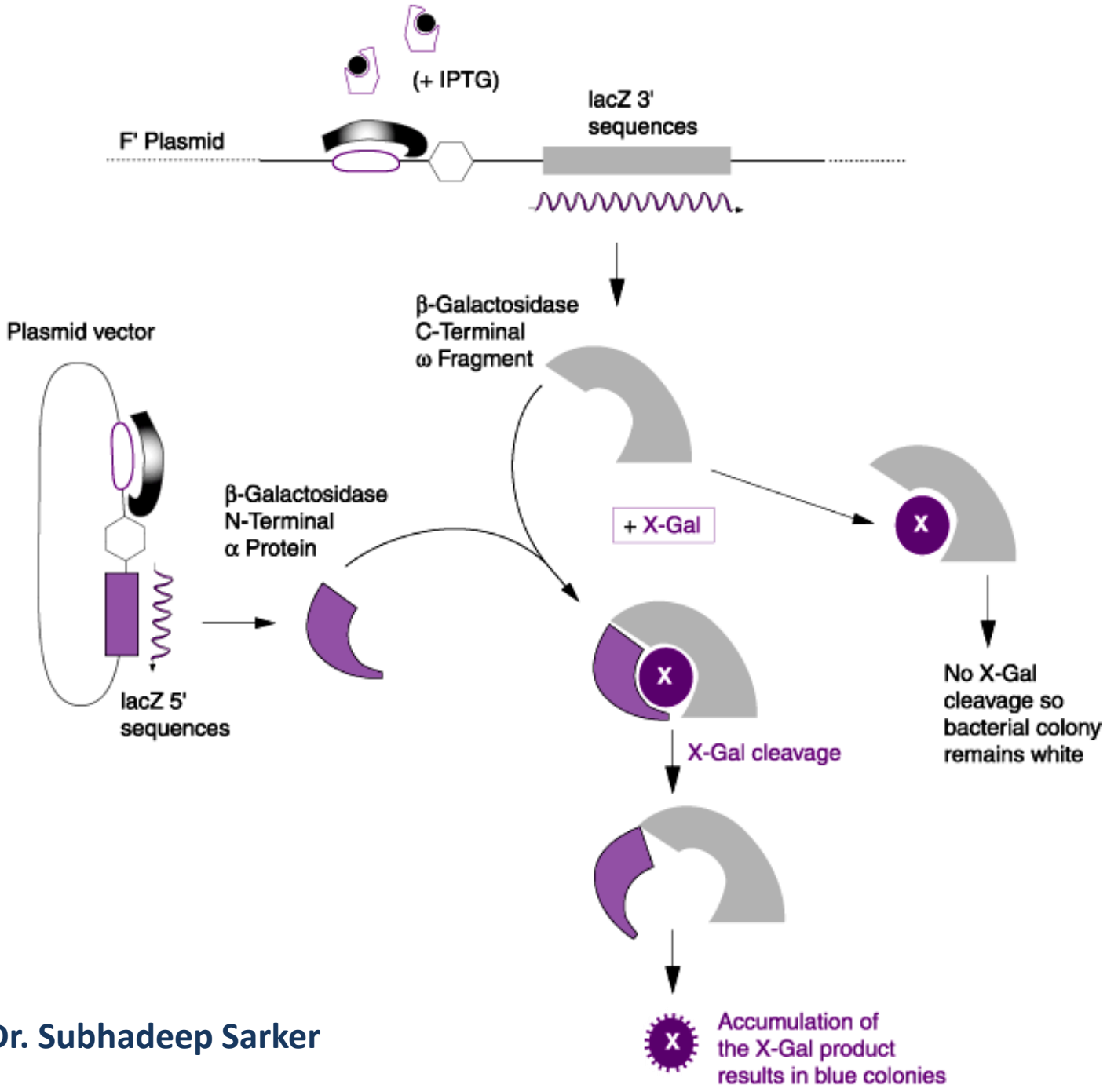
- Example of cloning vectors used for this test are pUC19, pBluescript, pGem-T Vectors, and it also requires the use of specific *E. coli* host strains such as DH5α which carries the mutant *lacZΔM15* gene.

Molecular Mechanism of α-Complementation

- β-galactosidase is an enzyme coded by the *lacZ* gene of *lac* operon in *Escherichia coli* and it exists as a homotetramer in its active state.

- A mutant β-galactosidase derived from the M15 strain of *E. coli* has its N-terminal residues 11—41 deleted and this mutant, the **ω-peptide**, is unable to form a tetramer and is inactive.

- However, this mutant form of protein can return fully to its active tetrameric state in presence of an N-terminal fragment of the protein, the α-peptide. When the two peptides are expressed together, they form a functional β-galactosidase enzyme.



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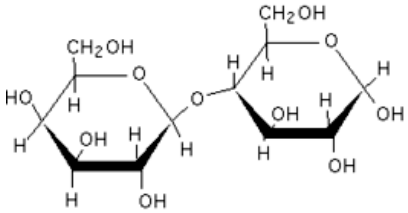
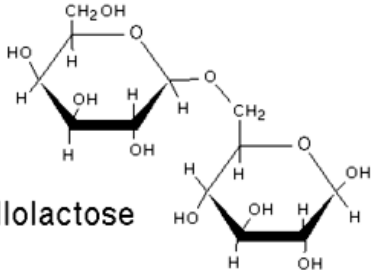
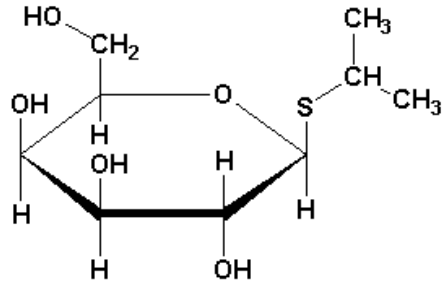
IPTG: The Inducer

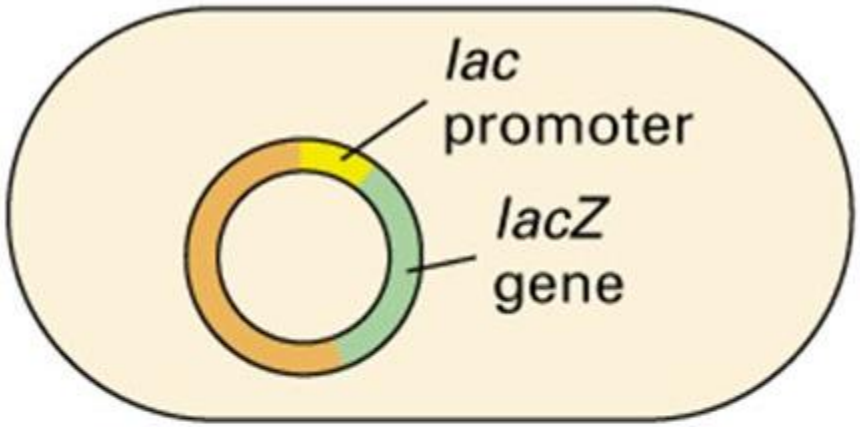


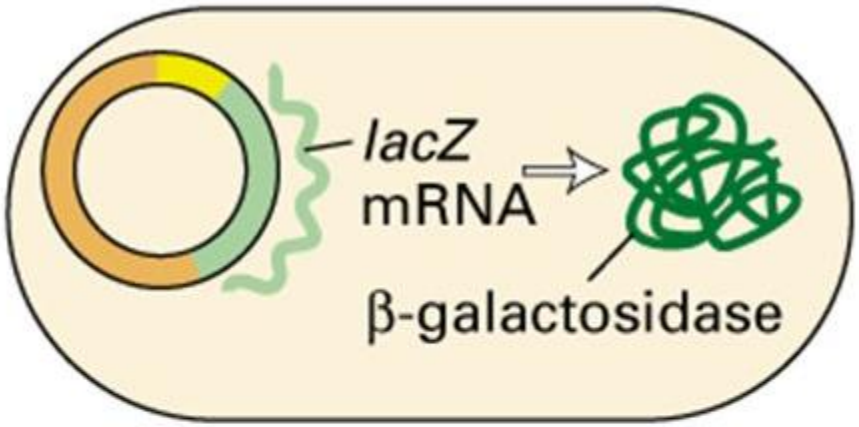




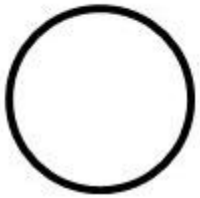
- **Isopropyl β-D-1-thiogalactopyranoside**, (IPTG) is a **molecular mimic of allolactose**, a lactose metabolite **that triggers transcription of the *lac* operon**.
- **Unlike allolactose, the sulfur atom creates a chemical bond which is non-hydrolyzable by the cell, preventing the cell from digesting the inductant; therefore the IPTG concentration remains constant.**

- In blue-white screening experiments, colonies that have been transformed with the recombinant plasmid rather than a non-recombinant one need to be identified. IPTG induces the transcription of the gene coding for β-galactosidase (*lacZΔM15*). If α-complementation does not occur and white colonies are produced, these cells are thought to be with the recombinant construct and therefore, the insert.
- **The advantage of IPTG for *in vivo* studies is that since it cannot be metabolized by *E. coli* its concentration remains constant. IPTG intake is also independent on the action of lactose permease, since other transport pathways are also involved.**



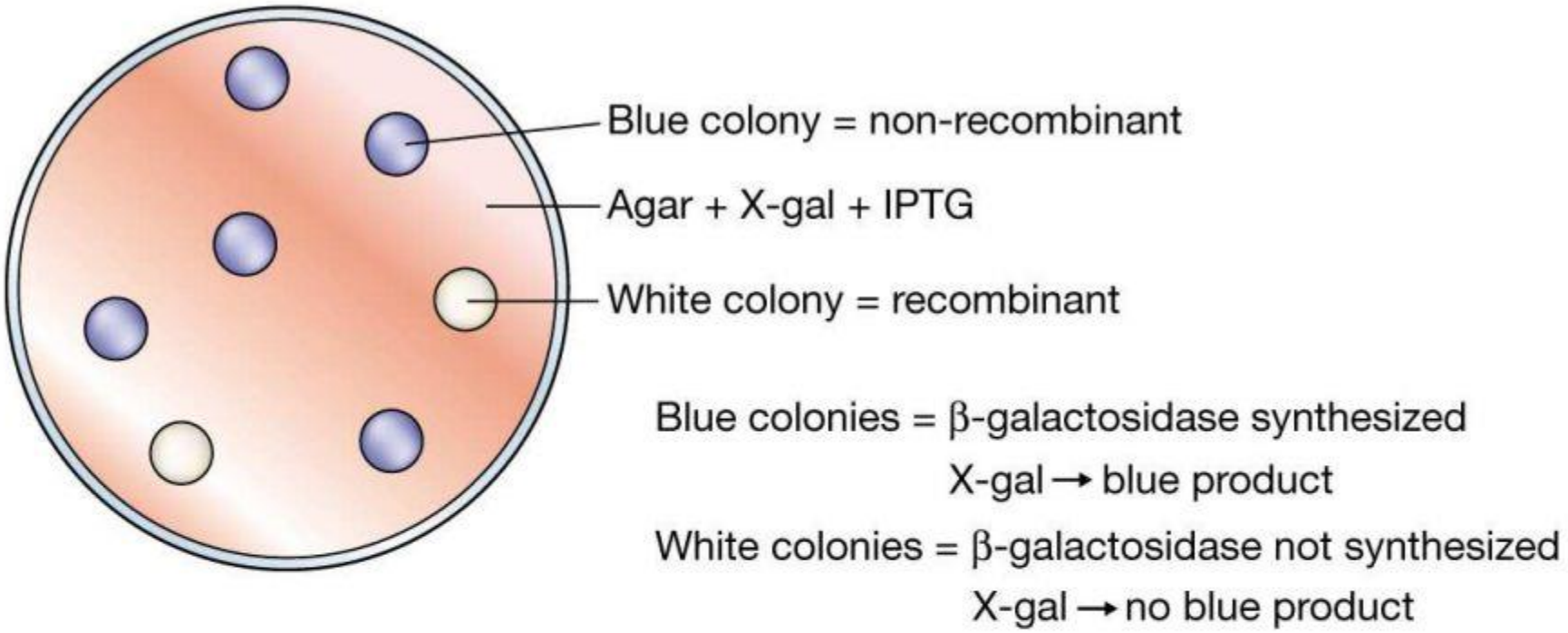


– IPTG
+ X-gal



+ IPTG
+ X-gal

(b) Screening for pUC8 recombinants

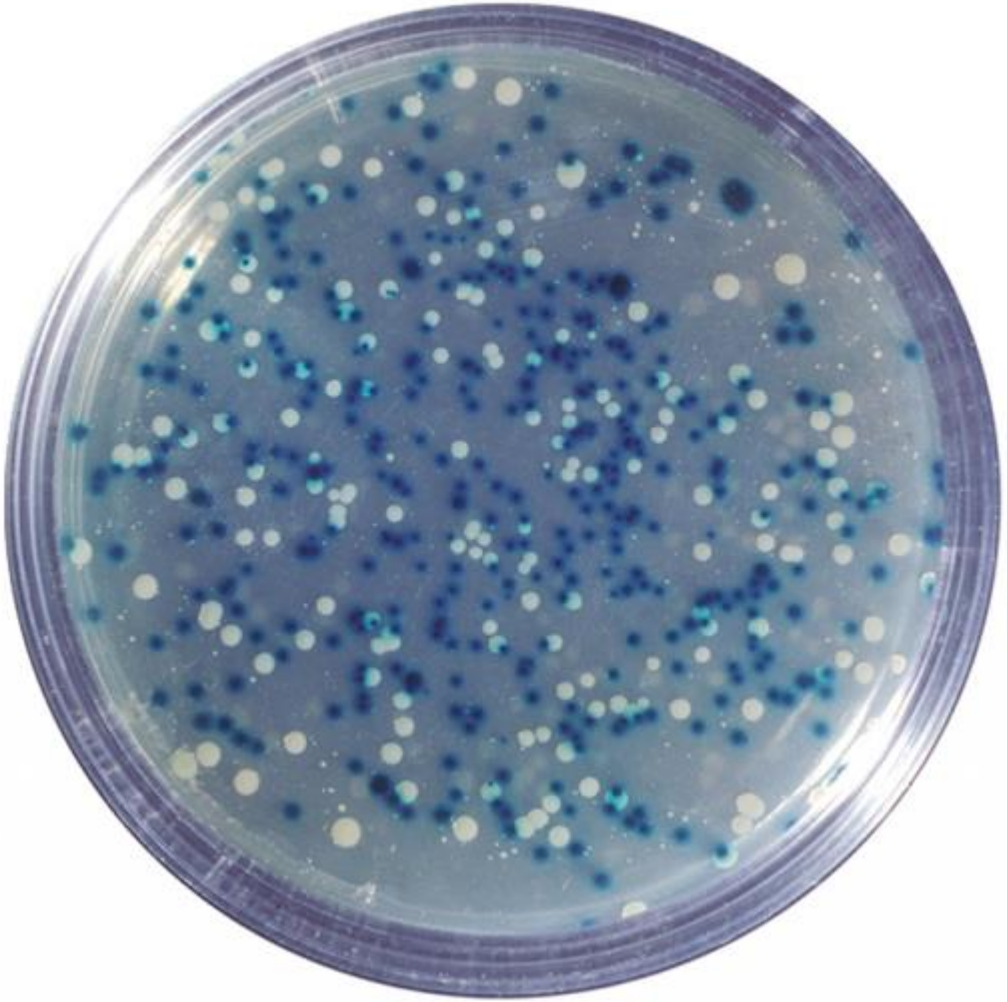


A lactose analog called **X-gal** (5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside) which is broken down by b-galactosidase to a product that is colored deep blue.

IPTG an inducer enzyme for X-Gal

This selection system is also called **Blue-White or Lac Screening**

X-gal identifies cells with beta-galactosidase



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